Notarangelo, Luigi 2021

Dr. Luigi Notarangelo Oral History

Download the PDF: Notarangelo_Luigi_oral_history (108 kB)

Dr. Luigi Notarangelo Oral history

Behind the Mask: May 4, 2021

GB: Good Morning. Today is May 4th, 2021. My name is Gabrielle Barr. I'm the archivist for the Office of NIH History and Stetten Museum, and today I have the pleasure of talking to Dr. Luigi Notarangelo. Dr. Notarangelo is the Chief of the Laboratory of Clinical Immunology and Microbiology and the Chief of the Immune Deficiency Genetics Section at the National Institute of Allergy and Infectious Diseases (NIAID). Today he's going to speak about some of his COVID research and experiences. Thank you very much for being with me.

LN: My pleasure.

GB: To start off, will you please describe the premise of your study that is looking at the composition of T and B cell receptors and mapping of virus-specific T-cell receptor sequences, or in simpler terms, how the adaptive immune system responds to COVID-19 over time?

LN: Sure. When the immune system has to fight against any infection, in this case specifically a viral infection, the long-term protection is actually provided by antibodies that bind to the virus as well as by one type of lymphocyte which is called T-cells, which can either kill virus-infected cells or help B cells make antibodies against the virus. The T and the B cells on their surface have molecules which we call T-cell receptors and B-cell receptors respectively, and those are molecules that recognize specifically the virus or viral proteins. This is the mechanism by which the immune system can specifically respond to a viral infection.

Our purpose was to sequence T-cell receptors and B-receptors, identify sequences of the T-cell receptor and B-cell receptor that are specific for the virus, in this case SARS-CoV-2 virus, and see how robust those T and B cell responses are and how durable they are. Another unknown question is for how long is immunity against this virus going to persist? We started this by looking at patients who actually got infected with COVID-19 and now we're also turning to individuals, not patients, but healthy individuals who have received the COVID-19 vaccine, because we want to make sure that the vaccine makes durable responses, both T cell response and antibody responses.

GB: Interesting. I had a follow-up question. In the case of SARS-CoV-2 do the T cells respond or has it been mostly the case of it causing the B cells to produce the antibodies?

LN: It's not an easy question to answer. Let me put it this way, it's much easier to measure antibodies against the virus than to measure T cell responses. Until recently it actually was very difficult to measure the responses of the T cells to the virus. There are ways to look at this. One way is, for instance, to stimulate the T cells with peptides, short fragments of proteins that are derived from the virus, and see whether the T cells become activated, whether they produce some molecules like interferon gamma.

That's one way of doing this but, of course, it's very laborious. It's time-consuming and it's expensive. Another way which is becoming very popular now is to sequence the T-cell receptor and again now there are very large databases that have collected information on sequences of the T-cell receptor that appear to be specific for some portion of the viral proteins. We can identify in that way basically T-cells are specific for the virus. Again it is expensive, and it may be some time-consuming, clearly not as easy as measuring antibodies to the virus, but it does provide evidence of T-cell immunity.

GB: Interesting. So what was your experience like in planning to get this very complex study underway?

LN: I should say that first of all, this is part of a collaboration we have with several centers in Italy and we at NIAID have created a consortium of investigators, multiple principal investigators, that look at the immune response to SARS-CoV-2 infection from different angles. My specific interest is in the adaptive immunity against the virus, so T and B cells, but other investigators have looked at inflammation, others are looking at the microbiome, others are looking at the genetics of the immune system and how [it may be] faulty. As you know gene defects may actually create susceptibility to severe COVID-19.

We've done this as I mentioned, in collaboration with several centers in Italy. Italy has been one of the countries in the world that has been hit the hardest by the infection and in particular we started this collaboration very early last year around February or March when Italy was one of the leading European countries, and actually leading world, in terms of number of cases. Some regions in Italy were particularly affected, in specifically northern Italy where I worked for many, many years. Together with Helen Sawyer and NIAID we initiated a collaboration with these centers in Italy and they were very eager to collaborate with us. They provided samples for my specific study.

Another very important component is a collaboration we had with industry, in particular Adaptive Biotechnologies, who actually performed the sequencing for us and then made the data available to us. We're analyzing the data on our own here at NIAID with the help of the bioinformatics group, Cihan Oguz and Justin Lack in particular. We also took advantage of the genetic effort underway at NIAID. As I mentioned, these samples were processed for all genome sequencing because the idea was to search for generic variants which may predispose to severe disease. As a byproduct of all this genome sequencing effort, we also could get information on the HLA, the human leukocyte antigen, constitution of these individuals and the peptides that are produced by the virus are presented to the immune system in a tight association with these actual molecules. There is what we call a restriction, so the genetic nature of the HLA dictates which peptides are presented better or less well to the immune system. So it's important to also know the HLA typing so we took advantage of all of this information and got very robust data now on the T-cell receptor repertoire of these individuals.

GB: That's really interesting. Can you talk a little bit in more detail about your methodology for your part of the study and the kinds of tools and equipment and technologies you and your team use?

LN: Yes, absolutely. What we receive from Italy is de-identified blood samples and they come in multiple types—we receive whole blood; we receive plasma or serum; we receive in some cases frozen peripheral blood molecular cells. Each of these types of samples is then made available to investigators here at NIH depending on their specific interest and their specific project. In my case, because my interest again is in the T and B responses, I've been collaborating with Peter Burbelo and Jeff Cohen in looking at antibody responses to two proteins of the SARS-CoV-2 virus, the so-called spike protein which is very important for allowing entry of the virus into the cells, and the nucleocapsid protein, the N-protein, that's done on plasma serum.

Also, in collaboration with colleagues across the street at Walter Reed (Dr. Andrew Snow and Clifton Dalgard), DNA was extracted there from whole blood samples using robotic machines. Some DNA was returned to us, and we shipped it to Adaptive Biotechnology. We used high-throughput sequencing technologies to amplify and sequence the T-cell receptor and the B-cell receptor rearrangements of these samples. The data were returned to us and in collaboration with bioinformatic people here in NIAID we're analyzing the T-cell receptor and B-cell receptor composition, mapping exactly the T-cell receptors that are specific for peptides or the virus and identifying exactly what is the frequency, what is the diversity of those T-cell receptors that are specific for the virus, and how do they evolve over time? For many individuals we actually have longitudinal samples that have been collected for up to nine months since the initial onset of the disease.

We have also collaborated with John Tsang here at NIAID. He and his team (in particular, Can Liu, a very talented PhD student) have analyzed the distribution of various blood cell types in patients with COVID-19 compared to healthy controls, and identified characteristic signatures of gene expression that distinguish patients with severe disease.

GB: I was just about to ask, were there any issues in terms of the samples when it comes time to evaluating data, like with examples maybe not being taken at the same time at a point of illness for a person, or things of that nature?

LN: Well obviously, as it's easy to imagine during a pandemic and especially when the hospital has lots of patients coming to the hospital. It is, in fact, very demanding for the physicians and for the nurses and for the technicians there to collect samples in the best possible way. But nonetheless, I have to say that our colleagues in Italy have done a wonderful job. They have used mostly discarded blood samples and they made themselves available working even after hours to process these discarded blood samples.

They have de-identified all of them and at the same time they have collected, and this is very important, clinical and laboratory metadata. Although we didn't have any information on the personal identifiers of these individuals, we knew pretty well what their trajectory had been during the admission. We knew which patients had been admitted and had required, for instance, admission to the intensive care unit, which patients eventually unfortunately died, which patients survived, what treatment they received, what were the main lab values. So, all of this was very precious information that was integrated with the research today, that was generated here at NIAID to correlate basically biomarkers of disease and how they are predictive or not of outcome in response to infection.

GB: Can you give a little bit of an idea of the time it takes to do that whole process that you were speaking of because it sounds like there are a lot of elements to this study?

LN: Well, it is not a straightforward process. First of all, you don't ship the samples one by one; you want to batch the samples. Just to give you an idea, we recently received a batch of samples from Brescia, Italy and it contained as many as over 2300 different samples. One has to appoint couriers to make sure that during the shipment all the samples are kept in dry ice. It's very important they're kept in dry ice because otherwise everything would be lost, so they have to refill the package and we're talking about many, many kilos—a hundred kilos of packages with dry ice. Then the package arrives here. People have to be available whenever the package arrives. Sometimes it has arrived over the weekend late in the evening, so people have to be available to receive the package, make sure that whatever is in the package is reflected in a manifest that we had received ahead of time. We doublecheck and if there are discrepancies, we inform our colleagues. They have to check and get back to us, and all of this has to be documented. Then samples have to be made available to the various investigators involved in the various research projects. We need databases where the presence and storage of these samples is integrated with the clinical and laboratory metadata that I mentioned before. Then we need help from bioinformaticians who handle and analyze big data. All of this takes a lot of time and coordination.

GB: How long does it take to clean up the data? Have you had issues with trying to make sense of all this information from different places?

LN: It's an ongoing process. It takes a lot of time, does require a lot of attention on our side, continuous conversation with our Italian colleagues because whenever we find discrepancies then we have to go back to them again. We don't have any personal identifiers, but they do, so they know exactly which code corresponds to whom. Errors may happen, but rarely. But a cleanup, of course, on our side is also very important. We need to make sure that we get consistent results when we analyze with the same method, the same sample, multiple types. So all of this is part of quality control.

GB: Can you talk a little bit about what your individual role has been and how your educational and professional background prepared you for being part of this COVID study?

LN: I've been coordinating this effort for NIAID along with Dr. Helen Su, who is also part of the same laboratory—Clinical Immunology and Microbiology—so the two of us have really set up everything. But again, none of this would have been possible without many, many other individuals who have helped through the process. This includes regulatory people here at NIH. Charles Rainwater has been our professional that has made sure that we were authorized to receive these de-identified samples and nothing was actually shipped before all of these were approved. In terms of my specific background, I'm a pediatrician. My main interest has always been in inborn errors of immunity, diagnosis, treatment, investigating the molecular and cellular basis of these diseases.

With Helen Su the main goal was to search for genetic variants that may also contribute to severe COVID-19. We are part of a large international network which is called the COVID-HGE, or COVID Human Genetic Effort, and the idea is to search for variants that may predispose to severe disease. We've already actually published genetic variants in 13 of these genes and there are more coming that predisposed to very severe, potentially life-threatening, COVID-19. We also internally here at NIAID and in collaboration with our colleagues at the Rockefeller University and in France with Dr. Holland here at the NIH, we've been able to identify also autoimmune mechanisms that contribute to severe COVID-19. I am referring to the presence of autoantibodies that neutralize the activity of some specific molecules called type 1 interferons. These type 1 interferons are produced by the cells in response to the viral infection. They restrict the capacity of the virus to replicate. If you neutralize the activity of the type 1 interferons, you block this restriction activity and so the virus comes unchecked, and it starts replicating within the cell, so you have more severe disease in individuals that have these neutralizing autoantibodies. In collaboration with Prof. Casanova at the Rockefeller, we found that as many as 15 percent of the patients with life threatening COVID-19 had these neutralizing autoantibodies. These autoantibodies are much more common in elderly individuals, and this may explain, at least in part, why COVID-19 is more severe in elderly subjects. It is possible that presence of these autoantibodies may also contribute to the risk of severe clinical outcome in other viral infections, such as influenza. There is still lot to be studied!

GB: What have been some of your other observations that you and your team have made this past year?

LN: With Dr. Lionakis and Dr. Kuhns we looked at biomarkers that may be predictive of severe outcome in COVID-19. Basically we analyzed the presence of 66 distinct proteins in the serum of the patients. Many of these are known to represent biomarkers of inflammation, so the idea was: Can we identify some biomarkers that when analyzed early in the course of the disease, shortly after admission to the hospital or shortly after having a positive PCR nasopharyngeal swab, are predictive of severe clinical outcome? We did identify several of them and four in particular whose levels remain significantly elevated throughout the course of hospitalization, and whose levels were clearly different in individuals who eventually die compared to those who survived. By analyzing the levels of these biomarkers shortly after admission to the hospital, it may be possible to identify subjects at higher risk of serious outcome, and therefore to treat these patients more aggressively.

We really didn't know at the beginning of this pandemic how to best treat these patients, but now we know when to use steroids; we know how and when to use some biological drugs. And there are monoclonal antibodies against the virus that are very efficacious. Having these biomarkers helps a lot .

GB: I was just going to ask you that, how your research translates to informing the care of patients with COVID-19?

LN: I think I answered this because we now know how to identify patients at higher risk. I should also say that there are some comorbidities that have been known for a long time, actually for more than a year, to be associated with poor outcomes. We know that having obesity, having cardiovascular disease, having some malignancies, would predispose [a person] to higher risk of severe infection. What we didn't quite know is whether these conditions or the use of immunosuppressive medications (and which ones of those) would also affect the capacity to respond to the virus and to develop immunological memory. In other words, are these individuals at risk of being re-infected with the same virus? Will they respond to the vaccine? We just published a paper in which we looked at patients with leukemias or lymphomas and noticed that in general they can mount a good antibody response with one exception: patients with lymphoma who had received rituximab, a drug that kills the B cells, within six months prior to the infection with the virus. This is because the lack of B cells made it impossible to these patients to make antibodies. But are they able to make T cell responses to the virus? This is what we are studying now. This will be very important to find out, because if this is true then these patients may benefit from the vaccine even if they are incapable of mounting antibody responses.

GB: What are some of the larger longer-term objectives of your study or any subsequent research you mentioned a little bit?

LN: We're interested in knowing what happens now in response to the vaccine in patients who are immune compromised, and I mentioned the patients with leukemia, I mentioned the patients who receive solid organ transplantation and require immunosuppressive drugs, but I also said that my main interest has always been in inborn areas of immunity. Here in NIAID, Dr. Emily Ricotta is in charge of a study which is listed in clinicaltrials.com. That study aims at evaluating efficacy and safety of the vaccine in patients with immune compromise, either genetic or acquired forms of immunocompromise. This is definitely something we're interested in.

The other thing that we're also interested in is to find out why children are in general less at risk of developing severe COVID-19, but a minor proportion of them develops a hyper-inflammatory disease called MIS-C (multi-system inflammatory syndrome in children). To study this, we have received samples from children with acute COVID-19 or MIS-C, and also from healthy children. We are studying the inflammatory response of these children, as well as the production of specific antibodies and T-cell responses to the virus, how treatment affects evolution of the disease, and how does all of this compare to what seen in adults

GB: When did you begin your studies with children? That's really interesting.

LN: Initially we focused our attention on adults, simply because it was in adults that the disease was reported for the first time in China in December 2019 and in Europe in January/February 2020. It took about a couple of months before the first cases of MIS-C in children were recognized and reported.

GB: That's really, really interesting. So what have been some personal challenges and opportunities for you that COVID has presented? It seems like you've been involved in a ton of different studies.

LN: Yes, challenges, many challenges. One of the challenges, for instance, was that we had to make sure that we abide the rules within NIH for being in the lab, so there was a time that only one person at a time was permitted to be in the lab. Actually, even for that we required special permission. It's not about the nature of the samples that we were working on because we were not culturing the virus. There was nothing particularly risky to the investigator, but we wanted to avoid crowding in the lab, so only one lab member at a time. Then things have become a little easier. We now can have multiple lab members at the same time but still maintaining social distancing even within the lab. That has been a challenge for us in terms of organizing all of these at a time when we're receiving all of these samples. It has been challenging for the lab members as it's also a stressful situation to have to come to work only at certain times of the day, on certain days of the week, and having to stop all other research projects that were ongoing at that time. This has affected all of us undoubtedly, but I have to say that it also has demonstrated incredible cohesion within our institute, so that actually enhanced the desire to collaborate with each other. This is not just unique to NIH. If you look at the literature, you will be amazed to see how many papers have been published in this period of time and how many of those papers actually are the result of international collaboration, like in our case. So really the scientists around the globe have experienced an unprecedented opportunity for collaborating with each other and talking with each other and finding solutions to an incredibly challenging problem.

GB: Do you ever get emotional given the nature of your work? Is it very sad dealing with these people who are very sick or who have died unfortunately? Also you're working so much with Italy and I know you're from Italy. Does that ever affect you emotionally?

LN: Not only I'm from Italy, but before moving to the United States I spent most of my career in Brescia, one of the cities that have been most heavily affected by COVID-19. For me, yes, it has been emotional to collaborate with people whom I knew very well, including physicians and nurses who were hospitalized with COVID-19 (some even in the ICU). I've lost friends, and so, for me working in collaboration with all of these has been very emotional. At the same time, I considered this a duty to my original country and a way to bring together Italy (the country where I'm from) and the United States (the country that has welcomed me and my family and where I work right now). I feel very grateful for having been part of this.

GB: That's very nice. We're going to end on a more fun question, a lighter note. What is one small way you've tried to preserve normalcy in a very uncertain environment?

LN: I'll tell you. I love chess. I have played when I was much younger for the young Italian national team of chess by mail. But I've never been a great chess player, though I dreamed I could be one. Recently a doctor from Estonia, now living in Finland, has joined my lab. I was pleased to see that she had played for the Estonian national chess team, and had competed with some top players, including the former female world chess champion. So, I brought my chessboard to the NIH. We play once a week and I enjoy doing this. I've won once, I've drawn many games, but lost quite a few. She's stronger than me but it doesn't matter. Playing with her has inspired me and given me again the pleasure of doing something I enjoyed doing when I was younger. And has given me some pleasure in such a difficult time.

GB: That's really nice. Is there anything else you would want to add as a clinician but also as a person living through the pandemic?

LN: I think we have learned a very important lesson in life. There is more that we should know about. Clearly, all of this is also related to how we treat our planet, and I would encourage everybody to read about this because it's not just about a virus. It's about what we do to our mother earth and I think it's important we treat our earth as best as we can. This is a duty as citizens if we want to limit the risk of new pandemics which undoubtedly will be there. We need to do our best to preserve our planet and minimize the risk of other pandemics.

GB: We can all agree to that. Well thank you very much and I wish you and your lab continued success. Stay healthy.

LN: Thank you, likewise. Thank you very much. It's been a pleasure.